

Article

Effect of Cornstalk Biochar Immobilized Bacteria on Ammonia Reduction in Laying Hen Manure Composting

Huaidan Zhang ¹, Jeremy N. Marchant-Forde ², Xinyi Zhang ¹ and Yan Wang ^{1,2,3,*}

¹ College of Animal Science, South China Agricultural University, Guangzhou 510642, China; zhd520888@163.com (H.Z.); zsz@stu.scau.edu.cn (X.Z.)

² Livestock Behavior Research Unit, USDA-ARS, West Lafayette, IN 47907, USA; jeremy.marchant-forde@usda.gov

³ Guangdong Provincial Key Lab of Agro-Animal Genomics and Molecular Breeding and Key lab of Chicken Genetics, Breeding and reproduction, Ministry of Agriculture, Guangzhou 510642, China.

* Correspondence: ywang@scau.edu.cn; Tel.: +86-20-85280279; Fax: +86-20-85280740

Received: 20 February 2020; Accepted: date; Published: 28 March 2020

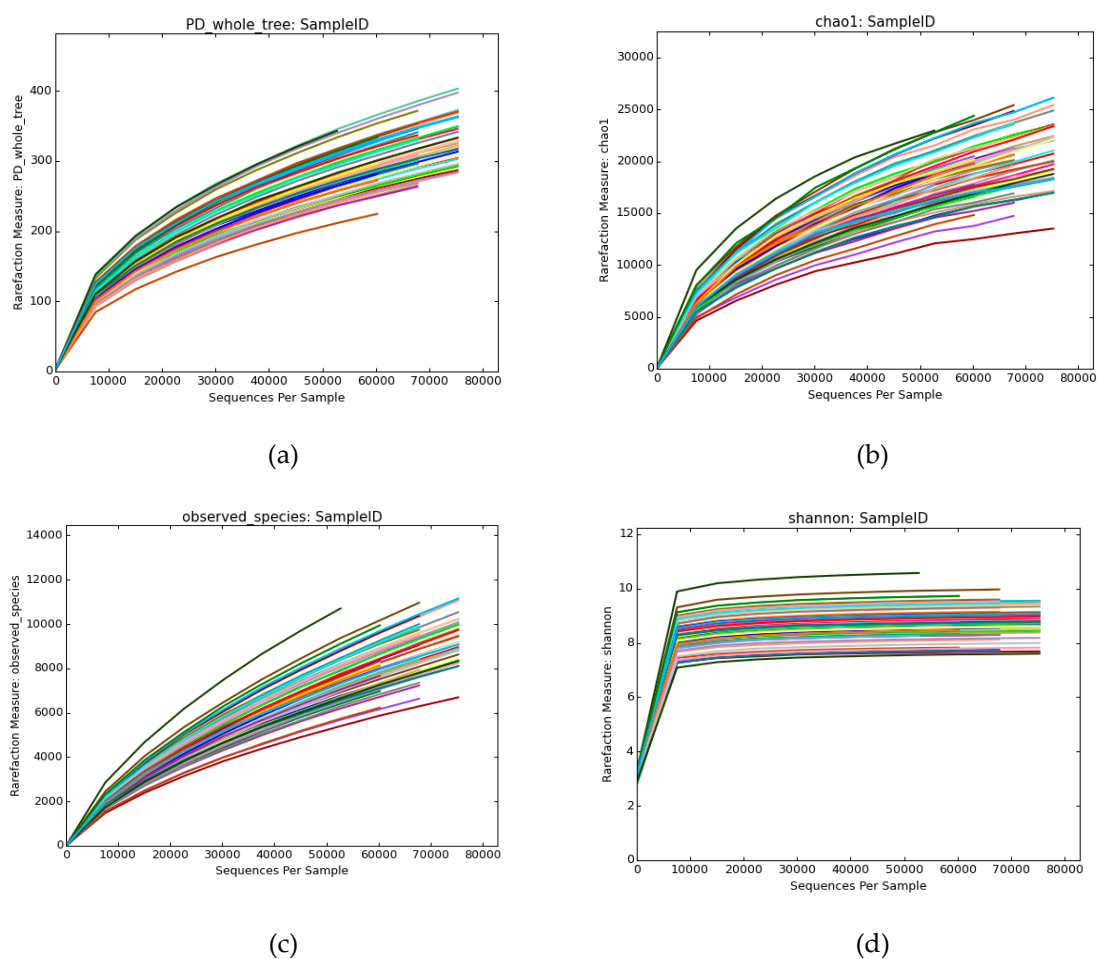
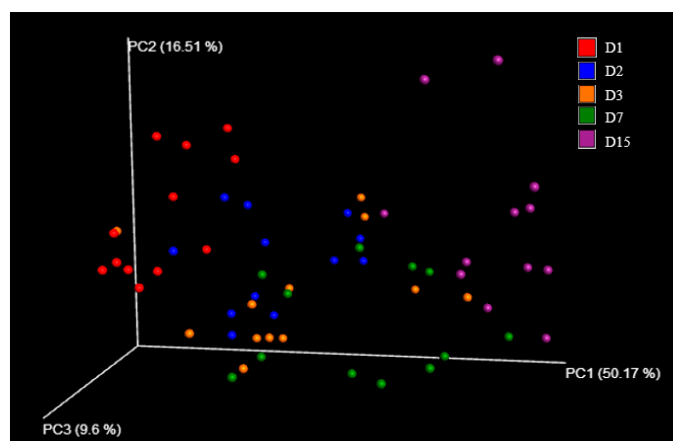
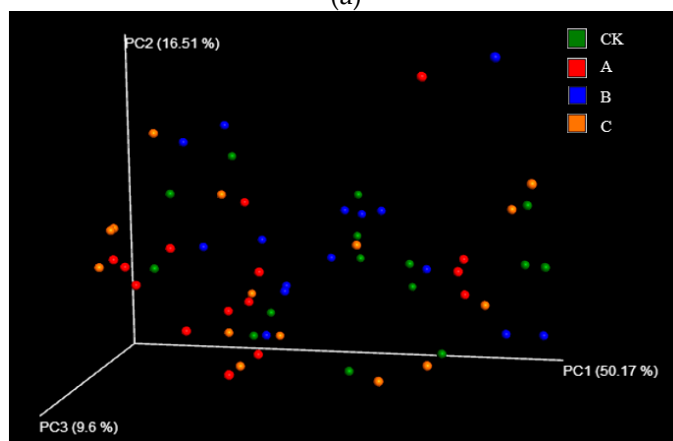


Figure S1. Alpha dilution curve of the sample; Note: a, b, c, d are PD, whole tree index, chao1 index, observed species index, shannon index dilution curve.



(a)



(b)

Figure S2. Sample cluster analysis; Note: (a), (b) are clusters analysis of samples, respectively, according to the days of composting and different treatment methods.

Table S1. Primary physicochemical characteristics of the raw materials (dry weight basis): laying hen manure, sawdust and biochar before composting.

	Moisture (%)	TOC (%)	TN (%)	C/N	pH	Surface area (m ² /g)	Pore volumes (cm ³ /g)
SB	8.65	73.52	0.82	89.66			
LM	65.77	34.69	4.23	8.20	8.78	68.25	0.42
SW	8.54	52.85	0.31	170.48			

¹ SB: straw biochar; LM: laying hen manure; SW: sawdust; TN: total nitrogen (based on dry weight); TOC: total carbon (based on dry weight).

Table S2. Bacterial loading rate.

	Pre-loading bacteria content (CFU/mL)	Bacteria content after loading (CFU/mL)	Bacterial loading rate (%)
<i>Bacillus stearothermophilus</i>	2.14 × 10 ⁸	7.89 × 10 ⁶	96.31
<i>Candida utilis</i>	1.99 × 10 ⁸	9.81 × 10 ⁶	95.07
<i>Bacillus subtilis</i>	2.53 × 10 ⁸	1.02 × 10 ⁷	95.97
Complex bacteria	2.22 × 10 ⁸	5.89 × 10 ⁶	97.35

Table S3. The specific primers for bacteria, fungi, *Bacillus stearothermophilus*, *Candida utilis*, *Bacillus subtilis*.

Microorganism	Primer sequences (5'-3')	Fragment size
Bacteria	F: ATTACCGCGGCTGCTGG	200bp
	R: CCTACGGGAGGCAGCAG	
Fungi	F: TCCGCAGGTTACCTACGGA	340bp
	R: GAGGCAATAACAGGTCTG	
<i>Bacillus stearothermophilus</i>	F: ACGGGAGGCAGCAGTAGGGA	389bp
	R: GCCTTCGCCACTGGTGTCC	
<i>Candida utilis</i>	F: TTACTACTTGGATAACCGTG	320bp
	R: GAGTCGCCTCCCGCCATAAG	
<i>Bacillus subtilis</i>	F: CCAACATCTCACGACACGAG	338bp
	R: AAGCAACGCGAAGAACCCTTA	

Table S4. The ordinary PCR reaction program for bacteria, fungi, *Bacillus stearothermophilus*, *Candida utilis*, *Bacillus subtilis*.

Step	Bacteria	Fungi	<i>Bacillus stearothermophilus</i>	<i>Candida utilis</i>	<i>Bacillus subtilis</i>
1. Initial denaturation	94 °C, 4 min	94 °C, 5 min	95 °C, 3 min	95 °C, 2 min	95 °C, 3 min
2. Denaturation	94 °C, 30 s	94 °C, 60 s	95 °C, 30 s	95 °C, 2 min	95 °C, 30 s
3. Primer annealing	63 °C, 30 s	53 °C, 60 s	52 °C, 30 s	95 °C, 15 s	60 °C, 30 s
4. Primer extension	72 °C, 72 s	72 °C, 60 s	72 °C, 30 s	58 °C, 35 s	72 °C, 30 s
5. Number of cycles	35	35	34	40	35
6. Final extension	72 °C, 10 min	72 °C, 10 min	72 °C, 7 min	72 °C, 10 min	72 °C, 10 min
7. Save	4 °C	4 °C	4 °C	4 °C	4 °C



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).